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APPLICATION NUMBER: ***60/107,006***
FILING DATE: ***November 04, 1998***

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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 C.F.R. § 1.53(c)	Docket Number:	018845.0158	Type a plus sign (+) inside this box→	+

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TITLE OF THE INVENTION (280 characters max)

Methods for the Production of TcRγδ⁺T Cells

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ENCLOSED APPLICATION PARTS (check all that apply)

X Specification Number of Pages 4 ___ Small Entity Statement

X Drawings Number of Sheets 4 ___ Other (Specify) _____

METHOD OF PAYMENT (check one)

A check or money order is enclosed to cover the Provisional filing fees.	PROVISIONAL FILING FEE AMOUNT
The Commissioner is hereby authorized to charge any additional filing fees and credit Deposit Account Number: 02-0375.	$

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

X No.

___ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE _____ DATE: November 4, 1998

TYPED OR PRINTED NAME James Remenick

REGISTRATION NUMBER (if appropriate): 36,902

_____ Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

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METHODS FOR THE PRODUCTION OF TcRγδ⁺ T CELLS

The following methods relate to the large scale, *ex vivo* expansion of TcRγδ⁺ T cells in liquid culture in the absence of a stromal layer. The starting material consists of low density mononuclear cells (LDMNC) from human peripheral blood. The LDMNC may be further fractionated by (1) enrichment for CD4⁺ T cells, (2) enrichment for T cells together with depletion of TcRαβ⁺ T cells, or (3) not further fractionated. The cells are cultured in medium containing conditioned medium (XLCM™), human sera or plasma (P), concanavalin A (con A), interleukin-2 (IL-2), and/or interleukin-4 (IL-4). At frequent intervals the cells are counted and reseeded with fresh medium, XLCM™, P, con A, IL-2, and/or IL-4. The percent of cells expressing a particular surface marker is determined using specific antibodies and flow cytometry.

Example 1. CD4e → XLCM™/P →→ TcRγδ⁺ T cells

Low density mononuclear cells were isolated from adult peripheral blood (LDMNC).

CD4⁺ cells were enriched from the LDMNC by negative selection using lineage specific antibodies and immunomagnetic affinity chromatography (CD4c).

The CD4e cells were expanded in culture medium containing 5% XLCM™ + 5% P.

The cells expanded more than 100,000-fold in four weeks.

After 21 days, more than 50% of the cultured cells were TcRγδ⁺.

The majority of the TcRγδ⁺ T cells were Vδ⁺.

See Figure 1.

Example 2. TeABd → XLCM™/P →→→ TcRγδ⁺ T cells

Low density mononuclear cells were isolated from adult peripheral blood (LDMNC).

T cells were enriched and TcRαβ⁺ cells were depleted from LDMNC by negative selection using lineage specific antibodies and immunomagnetic affinity chromatography (TeABd).

The TeABd were expanded in culture medium containing 5% XLCM™ + 5% P.

The cells expanded more than 100,000-fold in four weeks.

After 8 days, more than 50% of the cultured cells were TcRγδ⁺.

After 15 days, more than 80% of the cultured cells were TcRγδ⁺.

The majority of the TcRγδ⁺ T cells were Vδ⁺.

See Figure 2.

Advantages: The TcRγδ⁺ T cells expand more rapidly, expand to greater levels, and are more pure.

Example 3. LDMNC → XLCM™/P → IL-2/IL-4/P →→→ TcRγδ⁺ T cells

Low density mononuclear cells were isolated from adult peripheral blood (LDMNC).

The LDMNC were not further fractionated.

The LDMNC were expanded for 5 days in culture medium containing 5% XLCM™ + 5% P, following which they were divided, and half were continuously cultured in XLCM™/P, while the other half were washed and sub-cultured in 10 ng/ml IL-2 + 10 ng/ml IL-4 + 5% P.

In both cases, the cells expanded more than 100,000-fold in four weeks.

However, the different conditions gave rise to different kinds of cells.

Less than 5% of the cells cultured continuously in XLCM™/P were TcRγδ$^+$, while more than 50% of the cells cultured in XLCM™/P then sub-cultured in IL-2/IL-4/P were TcRγδ$^+$.

See Figure 3.

Advantages: The starting cell number can be very low since no initial fractionation is required. The sub-culture gets rid of XLCM™ components, e.g., con A, mezerein, other known or unknown factors.

Example 4. LDMNC → Con A/IL-2/IL-4/P → IL-2/IL-4/P →→→ TcRγδ$^+$ T cells

Low density mononuclear cells were isolated from adult peripheral blood (LDMNC).

The LDMNC were not further fractionated.

The LDMNC were expanded for 5 days in culture medium containing 20 ug/ml concanavalin A + 10 ng/ml IL-2 + 10 ng/ml IL-4 + 5% P, following which they were divided, and half were continuously cultured in concanavalin A + 10 ng/ml IL-2 + 10 ng/ml IL-4 + 5% P.

In both cases, the cells expanded more than 100,000-fold in four weeks.

However, the different conditions gave rise to different kinds of cells.

Less than 5% of the cells cultured continuously in XLCM™/P were TcRγδ$^+$, while more than 50% of the cells cultured in concanavalin A/IL-2/IL-4/P then sub-cultured in IL-2/IL-4/P were TcRγδ$^+$.

See Figure 4.

Advantages: The starting cell number can be very low since no initial fractionation is required. The culture conditions are completely defined (i.e., no conditioned medium).

We Claim

1. A method for the enrichment and culture of γδ+ T cells and cells produced by the method.

2. A method for the enrichment and culture of Vδ-1 T cells and/or Vδ-2 T cells and cells produced by the method.

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Figure 1

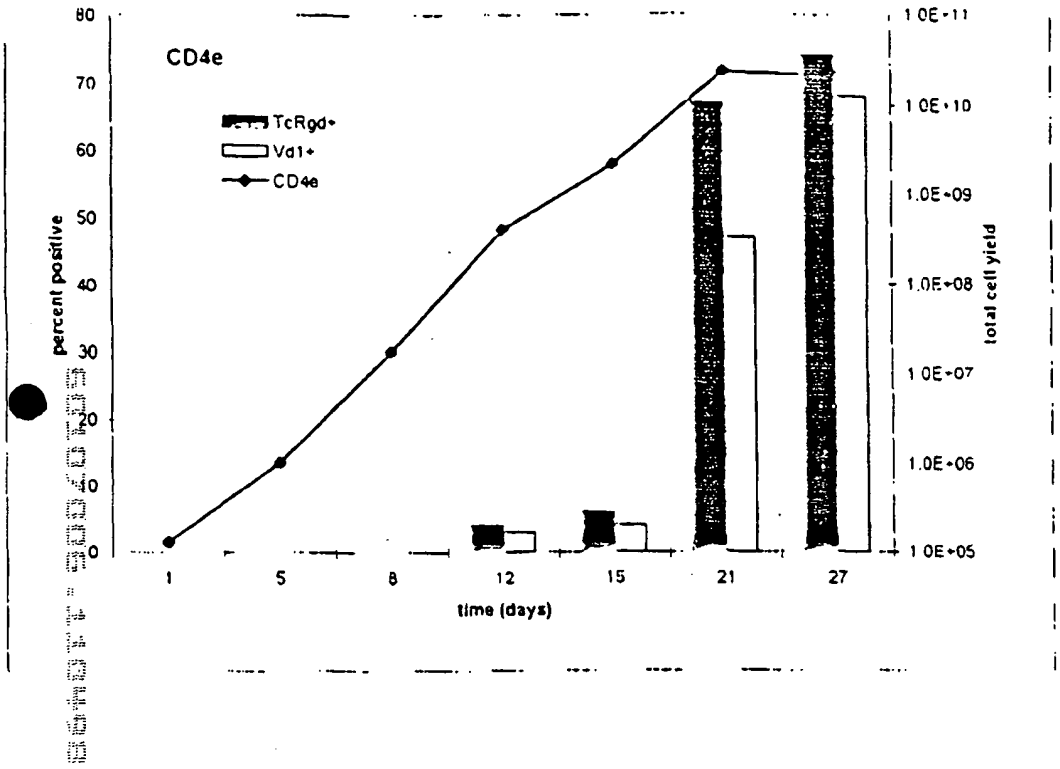

CD4e
TcRgd+
Vd1+
CD4e
percent positive
total cell yield
time (days)
80
70
60
50
40
30
20
10
0
1.0E+11
1.0E+10
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1
5
8
12
15
21
27

Figure 2

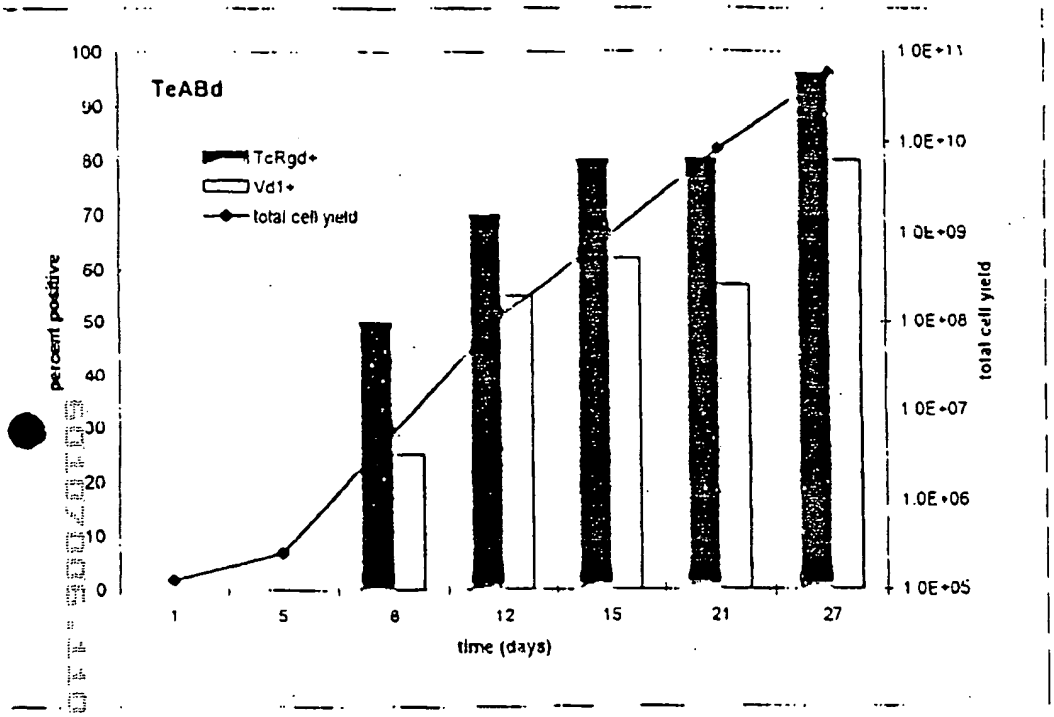

TeABd
TcRgd+
Vd1+
total cell yield
percent positive
total cell yield
time (days)
100
90
80
70
60
50
40
30
20
10
0
1.0E+11
1.0E+10
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1
5
8
12
15
21
27

Figure 3

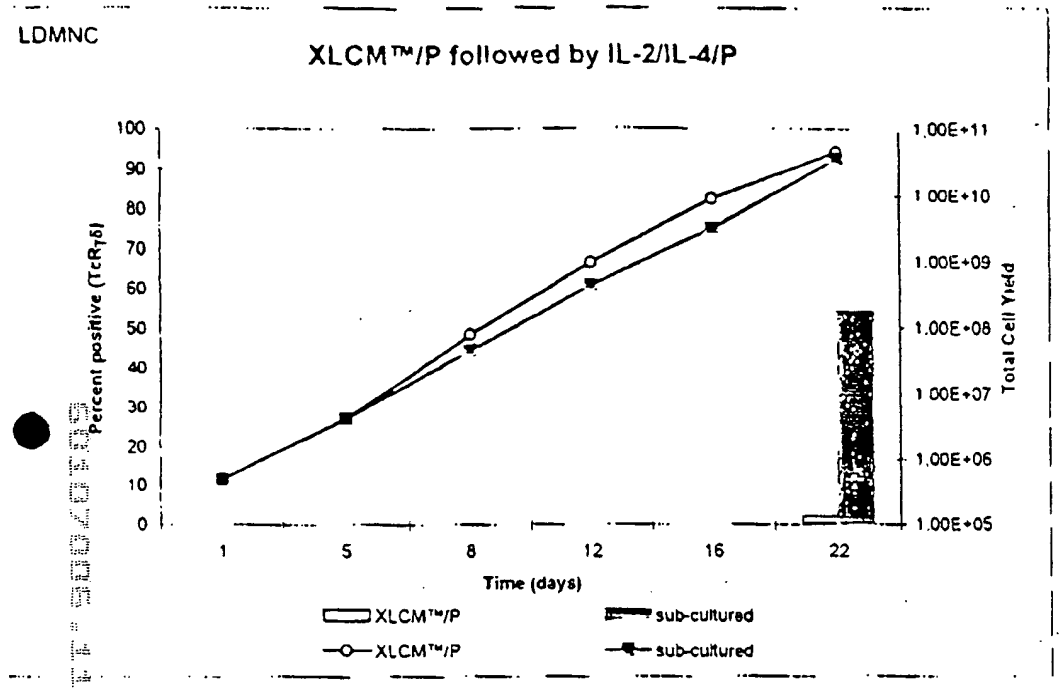
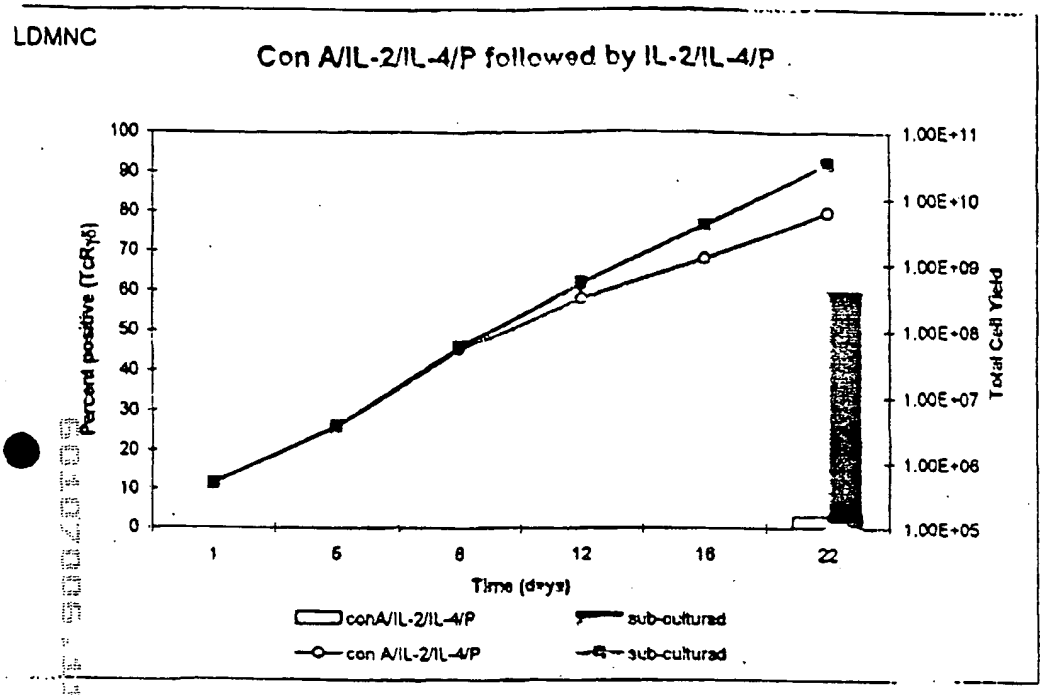

LDMNC
XLCM™/P followed by IL-2/IL-4/P
Percent positive (TcRγδ)
Total Cell Yield
Time (days)
XLCM™/P
sub-cultured
XLCM™/P
sub-cultured

Figure 4


LDMNC
Con A/IL-2/IL-4/P followed by IL-2/IL-4/P
Percent positive (TcRγδ)
Total Cell Yield
Time (days)
conA/IL-2/IL-4/P
sub-cultured
con A/IL-2/IL-4/P
sub-cultured